**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Applicant | : | Ivan N. Rich |
| Serial No. | : | 10/059,521 |
| Filed | : | January 29, 2002 |
| Title | : | High-Throughput Stem Cell Assay Of Hematopoietic Stem And Progenitor Cell Proliferation |
| Group Art Unit | : | 1641 |
| Examiner | : | Gabel, Gailene |

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## DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop **Amendment**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Ivan N. Rich, declare and state that:

1. I make this declaration in connection with U.S. application Serial No. 10/059,521. I am familiar with its prosecution history, particularly the Office Action mailed on February 27, 2006.

2. Attached is my Curriculum vitae Appendix A). In view of my education, training and experience, I consider myself, and am considered by my peers, to be an

expert in the field to which this application pertains and qualified to express opinions stated herein.

3. The Examiner alleges that one of ordinary skill in the art at the time of the instant invention would have been motivated to incorporate the culture system as taught by Bell and complimented with transferrin by Moore, which stimulates proliferation of hematopoietic cells in culture growth media, for subsequent use as MNC sample for testing proliferation status using the ATP bioluminescence assay as taught by Crouch, because methyl cellulose is conventionally known to advantageously increase viscosity of proliferating cells in culture media, and transferrin as taught by Moore is conventionally known to advantageously provide iron protein transport for cells in the media, and Bell specifically taught that erythroid progenitor colony formation is even further enhanced at lower, more physiological oxygen tensions, i.e., 5% oxygen; hence, increasing the concentration of hematopoietic progenitor cells for use in assays that measure proliferation of cell populations, including the ATP bioluminescence assay taught by Crouch.

4. The presently claimed invention is directed to a high-throughput stem cell assay of hematopoietic stem and progenitor cell proliferation.

5. Methyl cellulose is not "conventionally known to advantageously increase viscosity of proliferating cells in culture media". Methyl cellulose is an inert polymer that is used as a semi-solid and immobilizing medium so that when the cells are stimulated to proliferate and divide, they remain where they are and form colonies. Without the presence of methyl cellulose, one would have a so-called suspension culture, where the cells are free to move about the vessel and cannot form colonies of cells that can eventually be counted under a microscope. The whole point is that in the types of vessels presently used and for HALO performed in 96-well plates, the methyl cellulose allows the same conditions as the traditional colony-forming assay, but because we are measuring proliferation and not differentiation, the assay can detect intracellular ATP which is a limiting substrate for the luciferin/luiferase reaction, thereby obviating manual colony enumeration. The goal is to replace manual colony counting using a microscope with a non-subjective and fully standardized assay, thereby opening up many new applications.

6. Transferrin is not present in the medium as described by Moore, but is added as a mixture in BITSI. Unless, Moore's medium is registered under a different

name, I have never heard of COF medium and as far as I know, it is not even sold by any of the companies that provide media for tissue culture. Furthermore, the PCT WO 2004/018996 A2 published on 4 March 2004 and probably in the original submission, states on Page 22, lines 10-11, that iron-saturated human transferrin at a final concentration of $1 \times 10^{-10}$ mol/L or 0.1nM compared to 5 mg/L that Moore used for his COF media. These very low concentrations stem from my own previously published work (Rich IN, Sawatzki G & Kubanek B. Specific enhancement of mouse CFU-E by mouse transferrin. Brit. J. Haematol. (1981), 49:567-573).

7. Methyl cellulose does not "increase the viscosity of proliferating cells in culture media", and if it did, we would not have an assay system. Methyl cellulose is originally a solid substance that is dissolved in water and culture medium to produce a semi-solid, viscous fluid in which the cells are cultured. The purpose of the methyl cellulose is to immobilize the cells so that when they proliferate and divide, they remain where they are and do not move about in the culture. Only by this means can they form cell clusters and colonies. Thus, methyl cellulose increases the viscosity of medium in which the cells are grown, not the cells themselves.

8. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true. These statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 5/11/2006 Ivan N. Rich

Ivan N. Rich

## APPENDIX A
## CURRICULUM VITAE OF IVAN N. RICH PH.D.

### EDUCATION AND TRAINING

| INSTITUTION AND LOCATION | DEGREE | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| University of Sussex, Falmer/Brighton, | BSc | 1973 | Biochemistry |
| University of Ulm, Ulm, Germany | PhD | 1978 | Human Biology |
| University of Chicago, Chicago, IL, | Post Doc | 1981-1983 | Hematopoiesis |
| University of Ulm, Ulm, Germany | Habilitation | 1995 | Exp. Hematology |

### POSITIONS AND HONORS.

| | |
|---|---|
| 6/2000 – Present | Chairman & CEO of HemoGenix, Inc. |
| 4/1996 – 4/2000 | Director of Basic Research, Division of Transplantation Medicine, Palmetto Richland Memorial Hospital, Columbia, South Carolina and Adjunct Professor of Medicine, Department of Immunology and Microbiology, University of South Carolina School of Medicine. |
| 1/1983 – 4/1996 | Associate Professor and Director of the Experimental Hematology Laboratory, Division of Transfusion Medicine of the University of Ulm in the German Red Cross Blood Bank, Ulm, Germany. |
| 6/1981 – 12/1982 | Postdoctoral Fellow, Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois |
| 7/1973 – 6/1981 | Research Associate, Division of Transfusion Medicine, Department of Internal Medicine III, University of Ulm, Ulm, Germany. |

### SELECTED PEER-REVIEWED PUBLICATIONS


1. **Rich IN** & Kubanek B. Erythroid colony formation (CFUe) in fetal liver and adult bone marrow and spleen from the mouse. Blut (1976), 33:171-180.
2. Opitz U, Seidel H-J & **Rich IN**. Erythroid stem cells in Rauscher virus infected mice. Blut. (1977), 35:35-44.
3. Hansi W, **Rich IN**, Heit W, Kubanek B & Heimpel H. Erythroid colony-forming cells in aplastic anemia. Brit. J. Haematol. (1977), 37:483-488.
4. **Rich IN** & Kubanek B. The ontogeny of erythropoiesis in the mouse detected by the erythroid colony-forming technique. I.Hepatic and maternal erythropoiesis. J. Embryol. exp. Morph. (1979), 50:57-74.
5. **Rich IN** & Kubanek B. The ontogeny of erythropoiesis in the mouse detecetd by the erythroid colony-forming technique. II. Transition in erythropoietin sensitivity during development. J. Embryol. exp. Morph. (1980), 58:143-155.
6. **Rich IN**, Heit W & Kubanek B. The effects of actinomycin D on erythropoiesis. I. Short-term effects. Blut. (1980), 41:29-40.
7. **Rich IN** & Kubanek B. An erythropoietic stimulating factor similar to erythropoietin released my macrophages after treatment with silica. Blut. (1980), 40:297-303.

8. **Rich IN**, Sawatzki G & Kubanek B. Specific enhancement of mouse CFU-E by mouse transferrin. Brit. J. Haematol. (1981), 49:567-573.
9. **Rich IN** & Kubanek B. Release of erythropoietin from macrophages mediated by phagocytosis of crystalline silica. J. Reticuloendo. Soc. (1982), 31:17-30.
10. **Rich IN**, Heit W & Kubanek B. Extrarenal erythropoitein production by macrophages. Blood. (1982), 60:1007-1018.
11. **Rich IN** & Kubanek B. The effect of reduced oxygen tension on colony formation of erythropoietic cells in vitro. Brit. J. Haematol. (1982), 52:579-588.
12. Lappin TRJ, **I.Rich** & Goldwasser E. The effect of erythropoietin and other factors on DNA synthesis by mouse spleen cells. Exp. Hematol. (1983), 11:661-666.
13. **Rich IN**. Hemopoietic regulation in vitro: In vivo significance of functionally similar multiactive potentiating factors. Collection des Annales de l'Institut Pasteur, Annales D'Immunologie. (1984), 135C:280-288.
14. **Rich IN**. A role for the macrophage in normal hemopoiesis: I. Functional capacity of bone arrow macrophages to release hemopoietic growth factors. Exptl. Hemat. (1986), 8:738-745.
15. **Rich IN**. A role for the macrophage in normal hemopoiesis: II. Effect of varying oxygen tensions on the release of hemopoietic growth factors from bone marrow-derived macrophages in vitro. Exptl. Hemat. (1986), 8:746-751.
16. **Rich IN**, Editor: Molecular and Cellular Aspects of Erythropoietin and Erythropoiesis. NATO Advanced Science Institute, Springer Verlag. (1987).
17. **Rich IN**, Vogt Ch, Pentz S. Erythropoietin gene expression in vitro and in vivo detected by in situ hybridization. Blood Cells (1988) 14:505-520.
18. **Rich IN**. Haemopoietic regulation and the role of the macrophage in erythropoietic gene expression. Adv Exp Med Biol (1988) 241:55-66.
19. **Rich IN**. The macrophage as a production site for hematopoietic regulator molecules: Normal and pathophysiological signals. Anticancer Research. (1988) 8:1015-1040.
20. Vogt Ch, Pentz S, **Rich IN**. A role for the macrophage in normal hemopoiesis: III. In vivo and in vitro erythropoietin gene expression in macrophages detected by in situ hybridization. Exp Hemat (1989) 17:391-397.
21. Sawatzki G, **Rich IN**. Lactoferrin stimulates colony stimulating factor production in vitro and in vivo. Blood Cells (1989) 15:371-385.
22. Vogt Ch, Noé G, **Rich IN**. The role of the blood island during normal and 5-fluorouracil-perturbated hemopoiesis. Blood Cells. (1991), 17:105-125.
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29. **Rich IN**, Kubanek B. The autonomous release of erythropoietic inhibition during long-term in vivo administration of actinomycin D. Exp. Hematol. (1994) 22:347.
30. Kubanek B, **Rich IN**, Noé G. Erythropoietin. Infusionstherapie und Transfusionsmedizin. (1994) 21:46-50.
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35. Lappin TRJ and **Rich IN**. Erythropoietin - The first 90 years. Clin. Lab Hematol 18:137-145(1996).
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37. **Rich IN**. Standardization of the CFU-GM assay using hematopoietic growth factors. J. Hematotherapy:6:191-192 (1997).
38. Van Zant G, de Haan G, **Rich IN**. Alternative to stem cell renew from a developmental viewpoint. Exp. Hemat 25:187-192 (1997).
39. **Rich IN**, Brackmann I, Dewey MJ, Worthington-White D. Activation of the sodium/hydrogen exchanger via the fibronectin-integrin pathway results in hematopoiesis stimulation. J Cell Physiol. 177:109-122 (1998).
40. Noé G, Riedel W, Kubanek B, **Rich IN**. An ELISA specific for murine erythropoietin. Brit J Hematol 104:838-840 (1999).
41. Garden OA, Musk P, Worthington-White DA, Dewey M, **Rich IN**. Sequence comparison of the coding of human sodium/hydrogen exchanger isoform-1 cDNA in peripheral blood mononuclear cells of healthy volunteers and leukemic patients. Cancer Genetics and Cytogenetics 120:37-43 (2000).
42. **Rich IN**, Worthington-White D, Garden OA, Musk P. Apoptosis of leukemic cells correlates with reduction in intracellular pH after targeted inhibition of the $Na^+/H^+$ exchanger. Blood 95:1427-1434 (2000).
43. **Rich IN**. In vitro hemotoxicity testing in drug development. A review of past, present and future applications. Current Opinion in Drug Discovery and Development. 6:100-109 (2003).
44. Lis CG, Grutsch JF, Wood P, You M, **Rich IN**, Hrushesky WJM. Circadian timing in cancer treatment: The biological foundation for an integrative approach. Integrative Cancer Therapies. 2:105-111 (2003).
45. **Rich IN**. Stem cell haemotoxicity testing to predict toxic side-effects as a tool for safety monitoring during drug development. European Biopharmaceutical Review, Autumn, 2004.

**RESEARCH SUPPORT.**

1. SBIR Phase I entitled "An in vitro high throughput stem cell hemotoxicity assay" through the National Cancer Institute, 2001-2002 in response to the Program Announcement (PA-02-075), Innovative Toxicology Models. 2 R44 CA 93244-01

2. SBIR Phase II entitled "An in vitro high throughput stem cell hemotoxicity assay" through the National Cancer Institute, 2003-2005, in response to the Program Announcement (PA-02-075), Innovative Toxicology Models. 2 R44 CA 93244-02